Electrical methods for creating and characterizing biomolecular arrays for DNA hybridization and antibody detection at diamond surfaces

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Abstract

INTRODUCTION

Biologically-modified diamond presents a nearly ideal platform for integrating biological systems with microelectronics.[1-2] While most electrical biosensing schemes involve some type of amplification scheme such as the use of enzymes, in order to extend electrical biosensing to high-density arrays it is necessary to develop more general biosensing schemes in order to be able to detect a broader range of biomolecules without chemical interference between the different sensor elements. The use of the intrinsic *charge* of a biomolecule binding to a surface presents one such detection method. Since diamond is a wide-bandgap semiconductor, changes in charge distribution associated with biomolecules binding to a surface functionalized with specific, complementary biomolecular recognition elements provides a completely real-time, label free way to convert biological information into electrical control signals. We have been investigating the electrical properties of diamond surfaces modified with DNA and with antibodies,[3-5] as a basis for new types of direct electronic biosensor. Measurements have been made parallel to the interface and perpendicular to the interface in a Bio-FET geometry. We have also achieved the converse process, or using electrical control signals to induce specific molecular chemistry at the surface, as a way to perform electrically addressable preparation of diamond bioarrays without the need for microfluidics or other fluid-handling systems.[6-7] Thus, the unique electrical properties of diamond can be used both in the fabrication of bioarrays and in the direct detection of biological binding events.

ELECTRONIC BIOSENSING VIA IMPEDANCE AND BIO-FET MEASUREMENTS

Biomolecules interacting with semiconductors induce a "field effect" in which the change in local charge distribution induced by binding to the surface affects the diamond space-charge region. The impedance can be measured either perpendicular to the interface using AC impedance spectroscopy, or can be measured parallel to the surface in a field-effect-transistor geometry.[3-4] In AC impedance spectroscopy, only one electrical connection is needed to each sensing element; by choosing the AC frequency properly, one can capacitively couple across the molecular layer and achieve sensitivity to the diamond space-charge layer. Alternatively, one can place two electrical contacts directly onto the surface and measure the DC impedance changes induced in a diamond thin film.

To link biomolecules to the surface, we have developed a photochemical method that provides a very stable interface in which biomolecules of interest can be covalently linked to the diamond surface.[1] Upon hybridization, we observe a decrease in the impedance across the interface. Similar measurements made on modified silicon samples show a decrease in impedance on p-type samples and an increase in impedance on n-type samples. These results are all consistent with a field-effect, in which the negatively-charged DNA molecules induce an upward-band-bending and a corresponding decrease in resistance of the space-charge region.

` We have recently extended these measurements to true Bio-FET devices[4] in which we evaporate two contacts on top of the diamond. These contacts are then protected by evaporation of photoresist onto them (providing a hydrophobic barrier layer) while leaving the channel region open. We find that on a surface covalently

linked to the human antibody IgG, exposure to the corresponding antibody anti-IgG induces a significant decrease in drain-source current. Control experiment using a different antibody, anti-IgM, that is not expected to bind to the IgG-modified surface, shows no change in response. Thus, the diamond bio-FET responds selectively to the molecule of interest. There are many parallels between the parallel FET measurements and the perpendicular AC impedance measurements. The advantages and disadvantages of these two types of sensing will be discussed. However, both methods are useful for diamond-based biosensing arrays.

ELECTRICALLY-ADDRESSABLE FUNCTIONALIZATION OF DIAMOND FOR CREATION OF BIO-ARRAYS:

In order to create arrays of Bio-FET or other diamond-based sensing systems, it is necessary to develop methods for selectively functionalizing specific surface regions. While this has traditionally been done using mechanical "spotting" methods, we have recently developed a new method for electrically-addressable biomolecular functionalization of diamond surfaces. In this method, the diamond surfaces are first functionalized with aromatic nitro groups via a purely chemical method. The nitro groups on specific regions of the surface can then be selectively reduced to primary amines by applying a small potential of -1.5 V to the electrode of interest. These amine-functionalized surface regions can then be covalently linked to biomolecues of interest. We have used this method to functionalize diamond surfaces with specific DNA sequences in different areas without the use of microfluidics or other fluid-handling devices.

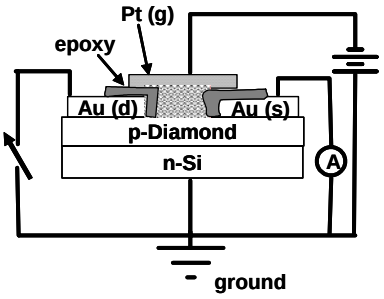

Figure 1: Diamond Bio-FET device

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